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A Mg^{++} DEPENDENT CORTICAL REACTION IN THE EGGS OF PENAEID SHRIMP (1)

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ABSTRACT   Mature penaeid oocytes contain large cortical special-
izations (rods) in their cortex. These rods reside in membrane
crypts and are isolated from the external environment by a thin
investment coat which surrounds the oocyte. When oocytes contact
seawater, at spawning, their cortical rods are expelled and form
a striking investment layer which subsequently dissipates. During
cortical rod dissipation a hatching membrane forms around the egg.
Oocytes spawned into Mg^{++} free seawater do not exhibit cortical
rod expulsion. If oocytes spawned into Mg^{++} free seawater are
returned to normal seawater cortical rod expulsion proceeds, how-
ever the expelled rods do not dissipate.

Mazia ('37) recognized that calcium played a significant role in the

cortical reaction of Arbacia punctulata eggs. The significance of calcium in

cortical reactions has been further documented in eggs of other echinoderms

and teleosts (Moser, '39; Yamamoto, '39, '54). Recent use of divalent cation

carrying antibiotics such as A23187 and X537A has confirmed the importance of

Ca^{++} ions in egg cortical reactions (Epel, '75; Steinhardt et al., '74; Stein-

hardt and Epel '74). Calcium is usually released from bound intracellular

stores. Similar dependence on Ca^{++} release for egg cortical reactions has

been demonstrated in amphibians (Schroeder and Strickland '74) and echiuroids

(Paul, '75). The Ca^{++} release is suspected of being involved in membrane

fusions (Schuel et al., '73b) and activation of proteases released from corti-

cal granules (Vacquier, '75a). In this paper we report the Mg^{++} dependence

of a massive cortical reaction in the oocytes of the white shrimp Penaeus

setiferus and the brown shrimp P. aztecus.

MATERIALS AND METHODS Both shrimp species were caught in otter trawls
in the Gulf of Mexico off Galveston, Texas. Animals were maintained at a
temperature of 18° C and a salinity of 34°/oo. The temperature was slowly
raised to 26° C over a period of 5 hours to induce spawning.

Microscopic observations were made on the cortical reaction of eggs spawned
into several media: natural seawater; artifical seawater lacking Mg^{++}; arti-
ficial seawater lacking Ca^{++}; artifical seawater lacking both Mg^{++} and Ca^{++}
(Mg^{++}-Ca^{++}); and natural seawater containing 1% procaine. Artificial seawaters
were prepared according to Cavanaugh ('56). Fifteen sets of experiments (12
with P. aztecus and 3 with P. setiferus) were run using these seawater systems.
A single animal was often used for each set of experiments since the animals
spawned large numbers of eggs (100,000 to 250,000). Eggs were collected by
holding a spawning female over beakers containing the seawater solutions.

Oocytes prepared for light and electron microscopy were fixed according
to the technique of Karnovsky ('65), dehydrated in acetone and embedded in a
low viscosity epoxy resin (Spurr, '69). Sections were cut and stained as de-
scribed by Dewel and Clark ('74).

RESULTS A mature, unreacted penaeid oocyte removed from the oviduct of
a spawning female is spherical and approximately 250 μ in diameter (fig. 1).
Radial striations apparent in the peripheral cytoplasm of such oocytes represent
large (10 μ in width by 40 μ in length) cortical specializations (cortical
rods) which lie in membrane crypts around the cortex of the oocyte (figs. 5,
6). A thin investment coat (1.3 μ) surrounds the oocyte and appears to be the
only structure separating the cortical rods from the environment (figs. 5,6).
When an oocyte is spawned into natural or artificial seawater, the investment
coat breaks down and the cortical rods are rapidly released into the surrounding

medium to form a large corona around the oocyte (fig. 2). Once completely expelled from the egg the rods begin to swell and dissipate quickly (fig. 3). A "hatching membrane" is formed around the egg several minutes (12-18) after the complete dissipation of the cortical rods (fig. 4).

Better than 99% of the oocytes spawned into Ca^{++}-Mg^{++} free seawater do not exhibit cortical rod release; however, oocytes spawned into Ca^{++} free seawater or seawater containing 1% procaine exhibit a normal cortical reaction and subsequent hatching membrane formation. It should be pointed out that the sequence of cortical events in the latter two solutions requires a slightly longer time (approximately 10%) for completion. As in the Ca^{++}-Mg^{++} free seawater, oocytes spawned in Mg^{++} free seawater do not exhibit any cortical reaction. When oocytes whose cortical reaction has been completely inhibited in Mg^{++} free seawater are returned to normal seawater, the rods are expelled from the eggs but do not dissipate and a hatching membrane is not formed. In all other respects the eggs returned to normal seawater from either the Ca^{++}-Mg^{++} free or Mg^{++} free seawater looked normal. At this stage, the cortical rods can be detached from the eggs by gentle agitation. However, this detachment promotes neither the dissipation of the cortical rods nor the formation of a hatching membrane.

DISCUSSION From the above data the cortical reaction of the penaeid egg can be divided into two distinct stages. These are (1) release of the cortical rods from the crypts in the cortex of the egg; and (2) dissipation of the cortical rods following release from the egg. Mg^{++} appears essential for rod release and may be required for rod dissipation although sufficient data are not available to clarify the latter assumption.

The Mg^{++} dependent release of the cortical rods from the shrimp oocyte is interesting since it is quite unlike the Ca^{++} dependent reactions reported

in other animal ova to date (Steinhardt and Epel, '74; Steinhardt et al., '74; Vacquier, '75b; Yamamoto, '39, '54). In other animal systems Ca^{++} is believed necessary for membrane fusion between the cortical granules and the oolemma during the cortical reaction. In penaeid oocytes, membrane fusion between the cortical rod crypts and the oolemma occurs in the ovary prior to spawning.

The dependency of the present cortical reaction on Mg^{++} is not understood. Whether Mg^{++} is required by a contractile system or is necessary for an enzyme system acting on the cortical specializations or the investment coat has not been determined. The release of enzymes during cortical granule breakdown in several different animal ova has been demonstrated by numerous workers (Epel et al., '69; Gwatkin et al., '73; Katsura and Tominaga, '74; Schuel et al., '73a, 73b; Vacquier and Epel '72). Other workers have suggested that enzymes released from cortical granules in sea urchins are activated by a divalent cation (Ca^{++}) released intracellularly at fertilization (Vacquier, '75a). In the present system Mg^{++} may have a similar function; that is, it may either activate an enzyme or act as a cofactor for an enzyme released upon egg contact with seawater.

FIGURE LEGENDS

Magnifications given are of micrographs submitted and not of journal's reduction of 20%.
1   Light micrograph showing an unreacted penaeid oocyte. X 110.
2   Light micrograph of a corona of cortical rods around a penaeid oocyte. X 110.
3   Light micrograph showing the dissipation of the cortical rods around the egg. X 110.
4   Light micrograph of a penaeid egg with a fully formed hatching membrane. X 110.
5   Light micrograph of a thick plastic section showing the cortical rods in their crypts and a thin investment coat surrounding the oocyte. Arrow-investment coat; CR-cortical rod. X 1225.
6   Transmission electron micrograph showing a rod in its crypt and the investment coat across the crypt. Arrow-investment coat; CR-cortical rod. X 8100.

1

2

3

4

CR

5

CR

6
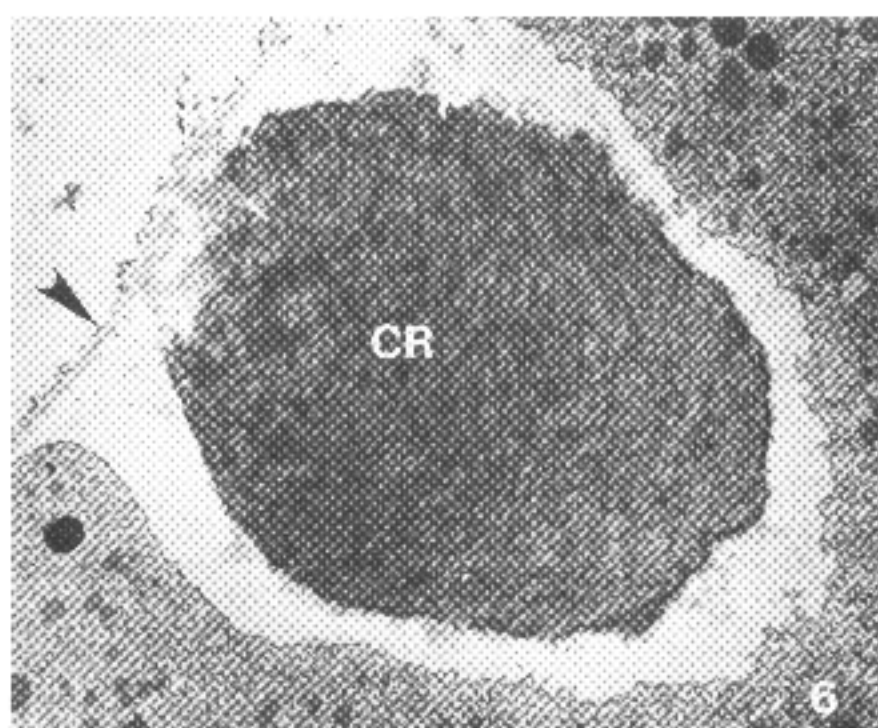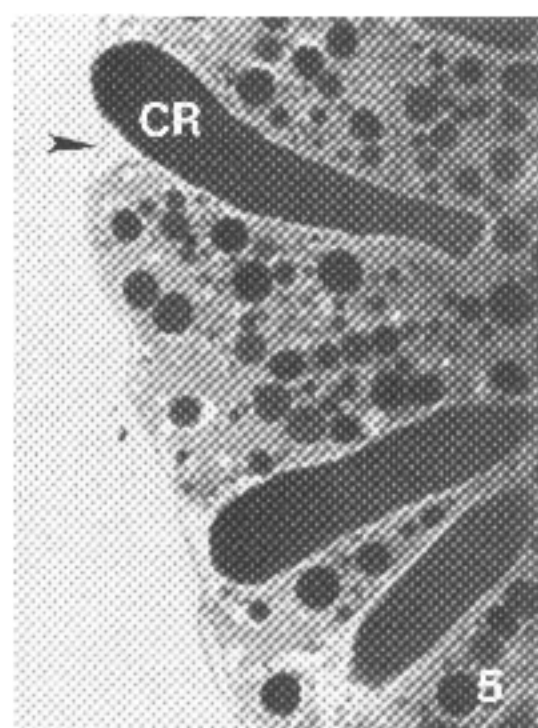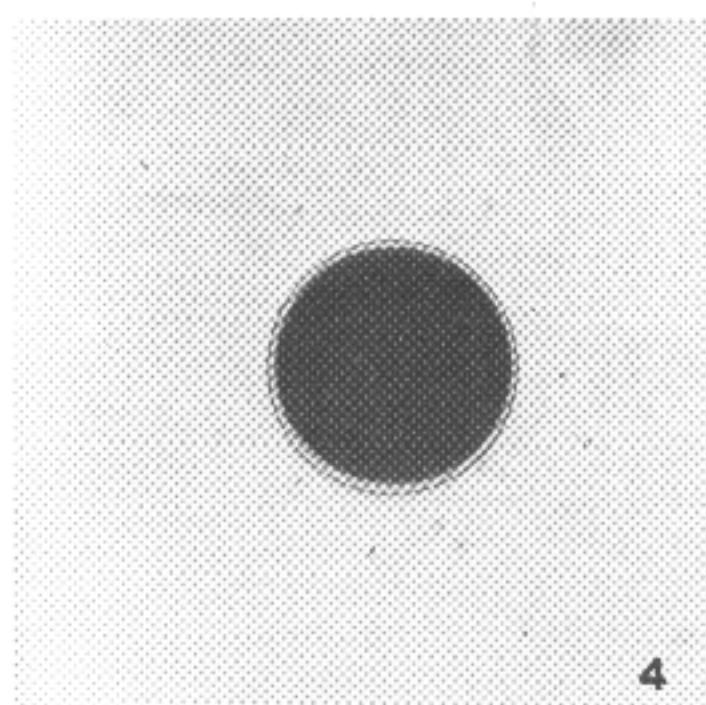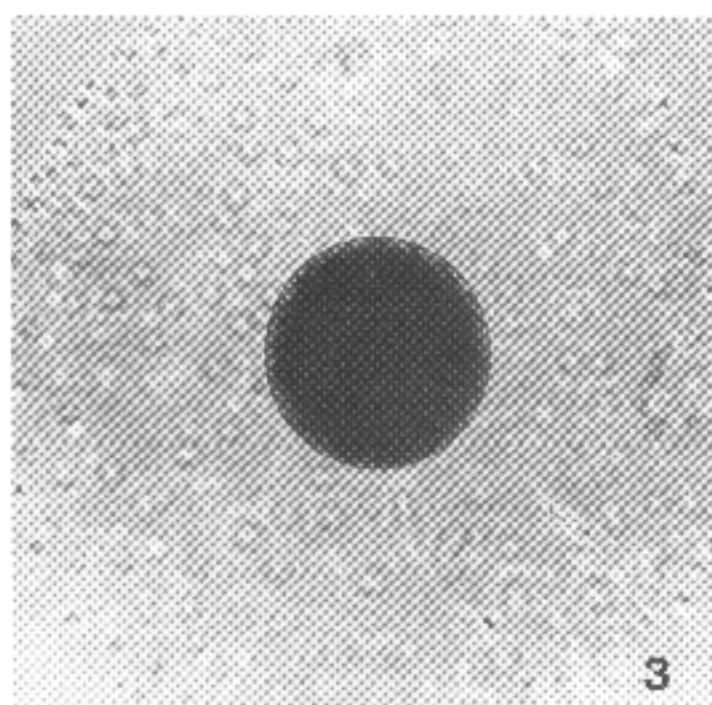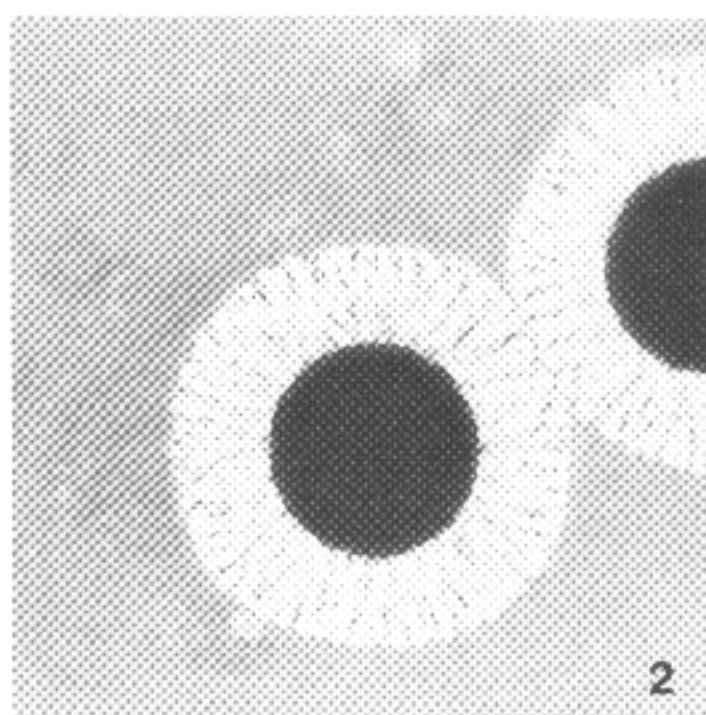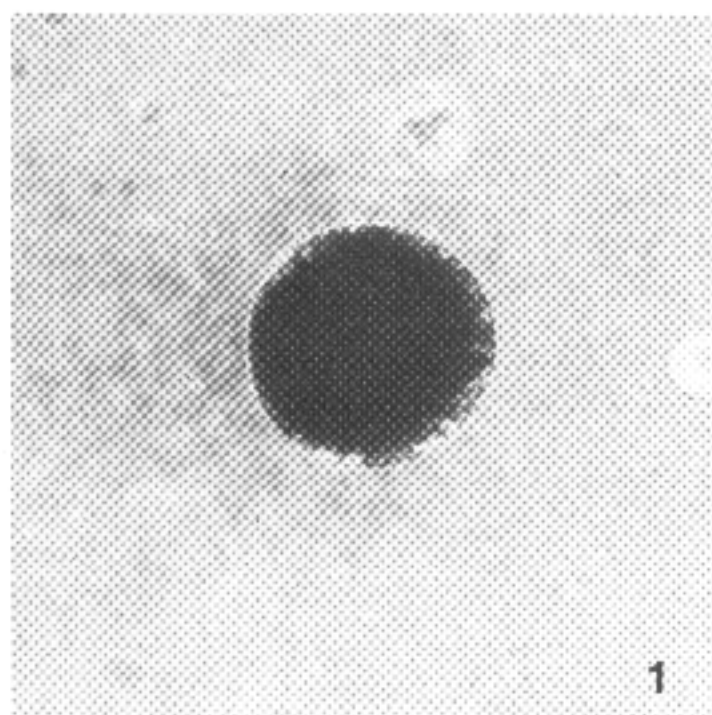
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